

A Randomized Study of a Single Dose of Intramuscular Cholecalciferol in Critically Ill Adults

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Objectives: To determine the effect of two doses of intramuscular cholecalciferol on serial serum 25-hydroxy-vitamin-D levels and on pharmacodynamics endpoints: calcium, phosphate, parathyroid hormone, C-reactive protein, interleukin-6, and cathelicidin in critically ill adults.

Design: Prospective randomized interventional study.

Setting: Tertiary, academic adult ICU.

Patients: Fifty critically ill adults with the systemic inflammatory response syndrome.

Intervention: Patients were randomly allocated to receive a single intramuscular dose of either 150,000 IU (0.15 mU) or 300,000 IU (0.3 mU) cholecalciferol.

Measurements and Main Results: Pharmacokinetic, pharmacodynamic parameters, and outcome measures were collected over a 14-day period or until ICU discharge, whichever was earlier. Prior to randomization, 28 of 50 patients (56%) were classified as vitamin D deficient. By day 7 after randomization, 15 of 23 (65%) and 14 of 21 patients (67%) normalized vitamin D levels with 0.15 and 0.3 mU, respectively ($p = 0.01$) and by day 14, 8 of 10 (80%) and 10 of 12 patients (83%) ($p = 0.004$), respectively. Secondary hyperparathyroidism was manifested in 28% of patients at baseline. Parathyroid hormone levels decreased over the study period with patients achieving vitamin D sufficiency at day 7 having significantly lower parathyroid hormone levels ($p < 0.01$). Inflammatory markers (C-reactive protein and interleukin-6) fell significantly over the study period. Greater increments in 25-hydroxy-vitamin-D were significantly associated with greater increments in cathelicidin at days 1 and 3 ($p = 0.04$ and 0.004 , respectively). Although in-hospital mortality rate did not differ between the groups, patients who did not mount a parathyroid hormone response to vitamin D deficiency had a higher mortality (35% vs 12%; $p = 0.05$). No significant adverse effects were observed.

Conclusions: A single dose of either dose of intramuscular cholecalciferol corrected vitamin D deficiency in the majority of critically ill patients. Greater vitamin D increments were associated with early greater cathelicidin increases, suggesting a possible

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mechanism of vitamin D supplementation in inducing bactericidal pleiotropic effects. (*Crit Care Med* 2015; XX:00–00)

Key Words: calcium; cholecalciferol; critical illness; hyperparathyroidism secondary; inflammatory markers; vitamin D

Several studies have reported a high prevalence of low vitamin D levels in critically ill patients ranging from 38% to 100% (1). This is associated with increased severity of acute illness, prolonged length of ICU length of stay, increased duration of mechanical ventilation, increased rates of infection, and an increased risk of death in hospital (2–7). The determination of vitamin D status assessment with subsequent enteral or parenteral administration is not a standard practice in ICUs. Patients in ICU typically only receive a small amount of vitamin D from standard nutritional formulations (\approx 200–400 IU) (8). Furthermore, for a variety of reasons, the delivery of adequate enteral nutrition in critical illness is challenging and consequently suboptimal (9). Due to potentially compromised gastrointestinal function in critical illness, oral doses of cholecalciferol may not be reliably absorbed. It is uncertain whether parenteral administration of vitamin D is more effective in restoring serum vitamin D in critical illness or whether this is associated with improved patient-centered outcomes. Currently, no licensed intravenous preparation of vitamin D is available apart from low-dose preparations (200–400 IU) used in multivitamin supplements. Given the unreliability of enteral absorption in the critically ill population, parenteral supplementation may be a more effective alternative for vitamin D repletion. There are little data about the pharmacokinetics, safety, and toxicity of parental vitamin D in critically ill patients. The biological impact of vitamin D replacement in critical illness is also uncertain.

The primary aim of this trial was to determine the effect of intramuscular dosing with two doses of cholecalciferol on levels on serial 25-hydroxy-vitamin D (25OHD) levels over a 14-day period. The secondary aim was to assess the effect of dosing on pharmacodynamics endpoints including calcium, phosphate, $1,25(\text{OH})_2\text{D}$, parathyroid hormone (PTH), cathelicidin (LL-37), and markers of inflammation, C-reactive protein (CRP) and interleukin-6(IL-6).

METHODS

Patients and Setting

Our study was a prospective, open-labeled, randomized trial conducted at an adult, university-affiliated, tertiary referral hospital in Sydney, Australia. Eligible patients were those who developed three of four criteria used to categorize the systemic inflammatory response syndrome (10) within 24 hours after admission and were expected to stay in ICU for at least 48 hours after randomization. Patients were excluded if they were pregnant, hypercalcemic (ionized calcium $>$ 1.3 mmol/L), had conditions associated with pathological 1-alpha hydroxylase activity such as sarcoidosis, lymphoma, or multiple myeloma,

had chronic kidney disease (estimated glomerular filtration rate $<$ 30 mL/min), or had a coagulopathy (platelet count $<$ 30,000 per mm^3 or international normalized ratio $>$ 3), thereby contraindicating an intramuscular injection.

We included all patients meeting inclusion criteria, rather than only vitamin D-deficient patients based on previous studies by our groups, showing that a single 25OHD level does not reflect vitamin D status in critical illness (11) and that 75% of our patient population had 25OHD levels of less than 50 nmol/L (7). In addition, the optimum level of 25OHD for its pleiotropic effects at tissue level is not known.

The cholecalciferol preparation used was Vitamin D3 Streuli 300,000 U (0.3 mU) commercially prepared by Streuli Pharma. Intramuscular injection was administered in the deltoid region with a 24-gauge needle, and the site was marked and observed for hematoma or infection over the study period.

The sample size of 25 patients per dose group was based on a previous study of a single dose of oral cholecalciferol, which would allow us to detect a 10 nmol/L rise in 25OHD from baseline with a power of greater than 80% and a $p <$ 0.05 (12). Patients were randomly allocated to receive one of the two doses of cholecalciferol (150,000 or 300,000 IU) as a single dose administered intramuscularly, using a random number generator.

The choice of dose was based on current practice and the recommendation from a systematic review of 30 studies of cholecalciferol suggesting single vitamin D doses of 300,000 IU or greater are most effective at normalizing vitamin D status, suppressing PTH activity for up to 3 months. The authors recommend that vitamin D doses greater than 500,000 IU should be used with caution to minimize adverse events, such as hypercalcemia, falls, and fractures (13).

The study was approved by the hospital Human Research Ethics Committee (HREC/12/SVH/322), and informed consent was obtained from the patient or the person responsible or healthcare proxy. Apart from cholecalciferol administration, other aspects of care did not differ between the two groups and was determined by individual clinicians blinded to the study drug allocation.

Data Collection

Demographic data including ICU admission diagnosis, source of admission, and severity of illness scores (Simplified Acute Physiology Score II [14], Acute Physiology and Chronic Health Evaluation II [15]) were collected. In addition, information on requirement for mechanical ventilation, vasoconstrictor and/or inotropic infusions, renal replacement therapy, and extracorporeal membrane oxygenation support was collected. History of vitamin D supplementation prior to critical illness was noted.

Pharmacokinetic Data

Serum 25OHD and $1,25(\text{OH})_2\text{D}$ concentrations were measured at baseline, day 1, day 3, day 7, and day 14 (or until discharge from the ICU, whichever was earlier) following administration of the cholecalciferol dose.

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Pharmacodynamic Data

Serum PTH, calcium, magnesium, and phosphate concentrations were measured at baseline, day 1, day 3, day 7, and day 14 following administration of the cholecalciferol dose. To further assess pharmacodynamic effects, LL-37 and inflammatory markers (serum CRP, and IL-6 levels) were measured at baseline, day 3, day 7, and day 14 following cholecalciferol dosing. Clinical outcome measures collected were duration of ICU and hospital stay or ICU and hospital mortality.

Assays

25OHD in nmol/L (1 ng/mL = 2.5 nmol/L) was measured using Liquid Chromatography Mass Spectrometry (Waters, Milford, MA) in a single reference laboratory (average coefficient of variation [CV] of the three quality control [QC] levels 6.2%). 1,25(OH)₂D was measured by Radioimmunoassay (Diasorin Cat No 65100E, Saluggia, Italy) (average CV of the three QC levels 22.4%). PTH analysis was performed by immunoassay with Beckman Dxi 800 immunoanalyser (Beckman Coulter, Brea, CA) (average CV of the three QC levels 6.6%).

Serum IL-6 was assayed by enzyme-linked immunosorbent assay (ELISA) (Human IL-6 ELISA Ready-SET-Go! kit; eBioscience, San Diego, CA). The lowest limit of detection was 2 pg/mL. Where necessary, serum samples were diluted with

assay diluent provided in the kit (CV Intra-assay, 4.6%; inter-assay, 4.7%).

LL-37 was assayed in the same serum samples by ELISA (HK321 Human LL-37 ELISA kit; Hycult Biotech Inc, Plymouth Meeting, PA) (CV intra-assay, 1.4%; interassay 12.9%).

Vitamin D deficiency was defined as 25OHD levels less than 50 nmol/L and severe deficiency as less than 30 nmol/L (16).

Statistical Analysis

Analysis was conducted with Stata 13.1 (StataCorp, College Station, TX) and Prism 6 (GraphPad Software, La Jolla, CA). Comparisons between randomized arms were made using a chi-square or Fisher exact test for categorical covariates, and a paired *t* test or Wilcoxon rank-sum test for continuous covariates, where appropriate. Formal comparisons of change in vitamin D sufficiency from baseline were made with McNemar test. Changes in vitamin D, laboratory parameters, and inflammatory cytokines from baseline were compared using mixed-effects random intercept model and used available data. Pearson correlation coefficients were used to assess the linear correlation between 25OHD and cytokines at baseline. Spearman rank correlation coefficient was used to assess the direction and relationship of change of 25-hydroxy D and cytokines from baseline to subsequent study days. *p* value of less than

or equal to 0.05 was considered statistically significant.

RESULTS

Fifty adult patients with the systemic inflammatory response syndrome were studied, of whom 72% were men with a mean (SD) age of 54 years (17.7 years). Twenty-five patients received a single dose of 0.15 mU of intramuscular cholecalciferol, and 25 patients received 0.3 mU. Patients were studied for the duration of their ICU stay resulting in the assessment of 25, 25, 23, 23, and 10 patients on day 0, 1, 3, 7, and 14, respectively, in the 0.15 mU group and 25, 24, 21, 21, and 12 patients on day 0, 1, 3, 7, and 14, respectively, in the 0.3 mU group (Fig. 1)

Demographic characteristics were balanced between groups (Table 1). None of the patients had liver failure. Five patients (three in the 0.15 mU and two in the 0.3 mU group) were on vitamin D supplements at a dose range of 1,000–3,000 IU/d prior to their admission for critical illness.

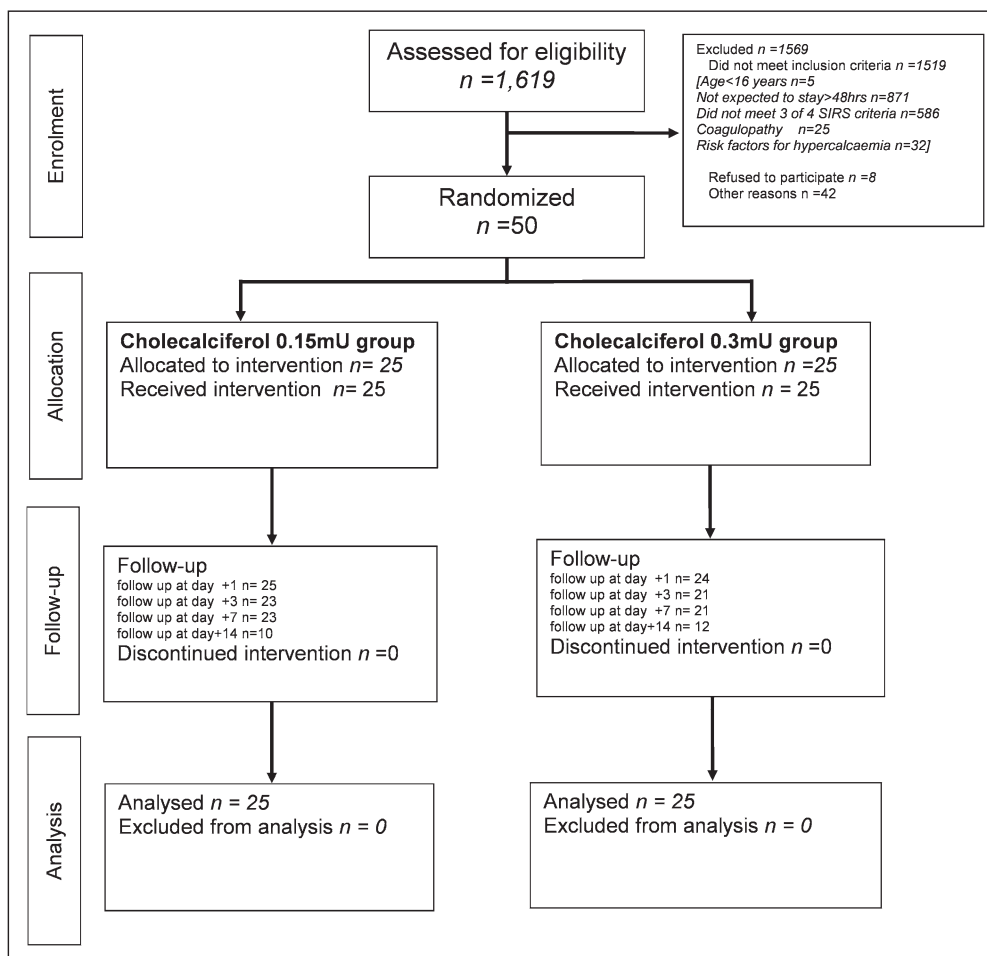


Figure 1. CONSORT diagram showing patient enrolment, allocation and follow-up. SIRS = systemic inflammatory response syndrome.

TABLE 1. Patient Characteristics at Baseline

Covariate	0.15-mU Dose (n = 25)	0.3-mU Dose (n = 25)	p*
Age (yr)	56 (43–63)	48 (43–71)	0.73
Male gender	16 (64)	20 (80)	0.35
Diagnosis			0.55
Trauma	10 (40)	7 (28)	
Sepsis	10 (40)	16 (56)	
Respiratory source	5/10 (50)	7/16 (44)	
Intra-abdominal source	2/10 (20)	5/16 (31)	
Other source	3/10 (30)	4/16 (25)	
Cardiogenic shock	4 (16)	1 (4)	
Other	1 (4)	1 (4)	
Acute Physiology and Chronic Health Evaluation II score	18 (16–23)	21 (16–25)	0.33
Simplified Acute Physiology Score II score	42 (32–53)	41 (36–55)	0.64
Mechanical ventilation	24 (96)	23 (92)	1.00
Renal replacement therapy	8 (32)	10 (40)	0.76
Extracorporeal membrane oxygenation support	6 (24)	6 (24)	1.00

*p values are derived from Wilcoxon rank-sum test for continuous covariates or Fisher exact test for categorical covariates.

Data are presented as median (interquartile range) for continuous variables and n (%) for categorical variables.

Baseline

Median (interquartile range [IQR]) 25-hydroxy D concentration of the entire group was 46.5 nmol/L (35–66 nmol/L). Overall, 56% of patients were vitamin D deficient, and 16% were severely deficient. Although median levels did not differ between the 0.15- and the 0.3-mU dose group (52 nmol/L [IQR, 40–67 nmol/L] vs 42 nmol/L [IQR, 32–62 nmol/L]; $p = 0.32$), 12 patients (48%) in the 0.15-mU group and 16 patients (64%) in the 0.3-mU group were vitamin D deficient ($p = 0.02$).

Of the 50 patients, seven (14%) were vitamin D deficient and had secondary hyperparathyroidism (PTH > 7 pmol/L), whereas seven (14%) had hyperparathyroidism in the absence of vitamin D deficiency. Renal function was normal in five of the latter group and affected in two as a part of their acute illness. All but one patient with hyperparathyroidism had low levels of ionized calcium (iCa < 1.15 mmol/L). Other baseline parameters and inflammatory biomarkers did not differ significantly between the two groups (Table 2).

Changes in 25OHD, 1,25(OH)₂D, and PTH

25OHD levels increased significantly in both groups from baseline (Fig. 2). The mean change from baseline in the two randomized groups was 11.2 nmol/L (4.3–18.1 nmol/L; $p = 0.002$) at day 3, 18.9 nmol/L (9.4–28.4 nmol/L; $p < 0.001$) at day 7, and 23.3 nmol/L (95% CI, 11.5 to 35.1 nmol/L; $p < 0.001$) at day 14. There was no significant difference in the mean change from baseline between the two groups over the entire study period (2.6 nmol/L; 95% CI, –6.3 to 11.5 nmol/L; $p = 0.57$).

The proportion of vitamin D-sufficient patients in the 0.15-mU group increased from 52% at baseline to 65% on day

7 ($p = 0.2$) and 90% on day 14 ($p = 0.2$) (Fig. 3A). The proportion of vitamin D-sufficient patients in the 0.3-mU group increased from 36% at baseline to 66% on day 7 ($p = 0.02$) and 83% on day 14 ($p = 0.008$) (Fig. 3B). Ten percent and 17% of patients remained vitamin D deficient by day 14 in the 0.15- and 0.3-mU groups, respectively. The proportion of severe vitamin D deficiency was 0% in both groups at day 14.

There was no change in 1,25(OH)₂D levels in the group as a whole ($p = 0.36$ and $p = 0.41$ at day 7 and day 14, respectively), and there was no difference between the two dose groups ($p = 0.23$).

There was a nonsignificant decrease in PTH at day 7 (mean change, –2.8 U; 95% CI, –6.0 to 0.36 U; $p = 0.08$) and a significant decrease from baseline to day 14 (mean change, –3.2 U; 95% CI, –6.0 to –0.39 U; $p = 0.03$), but the overall difference between the two groups over total follow-up was not significantly different (–1.7; 95% CI, –3.4 to 0.06; $p = 0.06$). However, those patients who were able to achieve a 25OHD level of greater than 50 nmol/L at day 7 had a significantly lower PTH level than those who were not able to achieve sufficiency (5.8 ± 5.3 vs 15.1 ± 17.9 nmol/L; $p < 0.01$).

Relation With Inflammatory Markers

CRP and IL-6 decreases were noted from day 3 and day 1, respectively (Fig. 4). The mean change in CRP from baseline was –43.9 mg/L (95% CI, –98.8 to 10.4 mg/L; $p = 0.11$) at day 7 and –74.4 mg/L (–112.4 to –36.4 mg/L; $p < 0.001$) at day 14. The difference between groups over total follow-up was not significant ($p = 0.53$). The mean change in IL-6 was –16.3 pg/mL (95% CI, –32.9 to 0.4 pg/mL; $p = 0.06$) at day 1; –36.3 pg/mL

TABLE 2. Median (Interquartile Range) Baseline Laboratory Parameters, by Randomized Arm

Parameter	0.15-mU Dose (n = 25)	0.3-mU Dose (n = 25)	p*
25-hydroxy-vitamin D (nmol/L)	52 (40–67)	42 (32–62)	0.32
1,25(OH) ₂ D (pmol/L)	124 (66–177)	98 (54–145)	0.35
Parathyroid hormone (pmol/L)	4.6 (2.8–7.5)	5.1 (2.5–8.2)	0.69
Ionized calcium (mmol/L)	1.09 (1.03–1.13)	1.15 (1.07–1.18)	0.01*
Magnesium (mmol/L)	0.91 (0.81–1.07)	0.94 (0.84–1.06)	0.82
Phosphate (mmol/L)	1.07 (0.72–1.33)	1.15 (0.95–1.31)	0.61
Creatinine (μmol/L)	97 (56–139)	84 (68–146)	0.95
C-reactive protein (mg/L)	156 (119–223)	109 (44–190)	0.12
Interleukin-6 (pg/mL)	60 (25–101)	47 (14–142)	0.94
Cathelicidin (ng/mL)	83 (45–181)	52 (38–162)	0.82

*p is a comparison of the laboratory parameter distribution between randomized arms using a Wilcoxon rank-sum test.

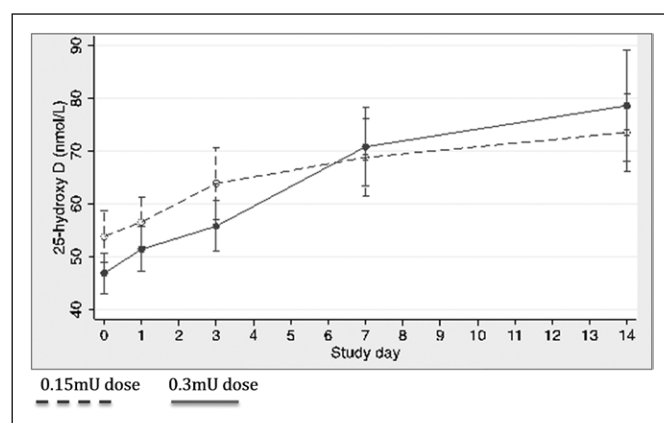


Figure 2. 25-hydroxy-vitamin-D increment over time in 0.15 and 0.3 mU groups. Error bars represent 1 SE.

(95% CI, –52.9 to –19.6 pg/mL; $p < 0.001$) at day 3; –49.2 pg/mL (95% CI, –66.1 to –32.2 pg/mL; $p < 0.001$) at day 7; and –52.0 pg/mL (95% CI, –78.0 to –26.0 pg/mL; $p < 0.001$) at day 14. Again, the difference between randomized groups over the study period was not significantly different ($p = 0.86$).

At baseline, 25OHD correlated positively with LL-37 (Pearson $\rho = 0.03$) (Fig. 5) and negatively with IL-6 (Pearson $\rho = -0.17$) although these were not statistically significant. Using generalized estimating equations, we found negative relationships between 25OHD concentration changes and biomarker changes over follow-up; however, only the relationship between 25OHD and IL-6 was significant (coefficient, –0.072; 95% CI, –0.10 to –0.04; $p < 0.001$) (Fig. 4). Although there was a negative but nonsignificant relationship between 25OHD and LL-37 over total follow-up, greater 25OHD increments in the early phase of ICU admission (day 1–3) were associated with larger increases in LL-37. Spearman ρ for the change in 25OHD versus the change in LL-37 to days 1 and 3 were 0.33 ($p = 0.04$) and 0.46 ($p = 0.004$), respectively (Fig. 5). This relationship was lost during the late phase of ICU admission (days 7–14).

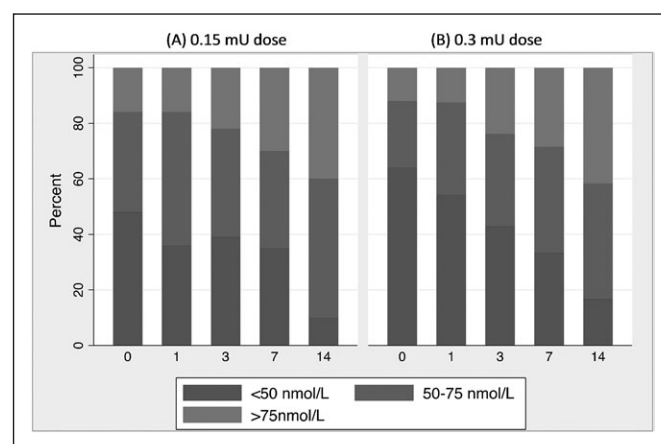


Figure 3. Proportion of patients in 25-hydroxy-vitamin-D level categories (in nmol/L) over time in the 0.15 and 0.3 mU groups. **A**, Number of subjects in 0.15-mU group D₀ n = 25, D₁ n = 25, D₃ n = 23, D₇ n = 23, D₁₄ n = 10; **(B)** number of subjects in 0.3-mU group D₀ n = 25, D₁ n = 24, D₃ n = 21, D₇ n = 21, D₁₄ n = 12.

Safety

One patient in the 0.15-mU group had ionized hypercalcemia with iCa of 1.46 mmol/L (RR, 1.15–1.3 mmol/L) on day 3, which returned to normal by day 7. Three patients (one in the 0.15-mU group and two in the 0.3-mU group) had mildly elevated iCa levels (between 1.3 and 1.4 mmol/L) over the study period. No patient developed total hypercalcemia (serum calcium > 2.6 mmol/L) during the course of the study. Hypervitaminosis D (25OHD > 200 nmol/L) was not observed at any time during the study period. No complications related to intramuscular injection were observed.

Outcomes

There was no statistically significant difference in mortality and hospital length of stay between the groups (Tables 3 and 4). However, vitamin D-deficient patients without hyperparathyroidism at baseline had a higher ICU and hospital mortality rate than vitamin D-deficient patients with secondary hyperparathyroidism (35% vs 12%; $p = 0.05$).

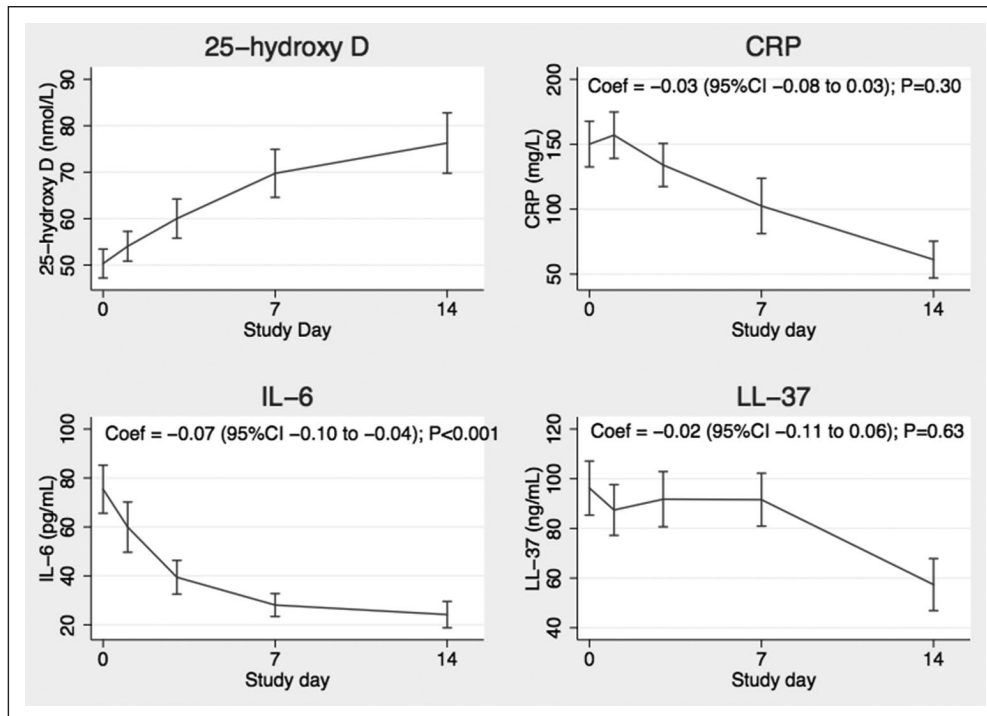


Figure 4. Change in 25-hydroxy-vitamin D (25OHD nmol/L), C-reactive protein (CRP mg/L), interleukin-6 (IL-6 pmol/mL), and cathelicidin (LL-37 ng/mL) over time. Error bars represent 1 se. Coefficients and 95% CI represent the estimated change in biomarker, per unit change in 25OHD over total follow-up.

DISCUSSION

Main Findings

A single dose of intramuscular cholecalciferol of either 0.15 or 0.3 mU corrected vitamin D deficiency in critically ill patients, in the absence of adverse effects. Vitamin D repletion was accompanied by a reduction in proinflammatory (IL-6) and induction of the antimicrobial peptide, LL-37 responses during the early phase of critical illness.

Comparison With Other Studies

Van den Berghe et al (17) ($n = 22$) investigated changes in 25OHD levels following administration of intravenous cholecalciferol at either 200 or 500 IU daily. Neither regime achieved vitamin D sufficiency. Mata-Granados et al (18) ($n = 11$) demonstrated that two oral doses of 60,000 IU of 25OHD in 11 critically ill patients significantly increased 25OHD levels and was able to correct vitamin D deficiency in 90% of patients. Amrein et al (19) administered 540,000 IU oral vitamin D in 10 critically ill patients and eight patients achieved normalization of levels. These studies included small patient populations. In the subsequent well-conducted randomized trial VITdAL of cholecalciferol supplementation, Amrein et al (19) demonstrated that 52% of the 237 patients who received cholecalciferol supplementation with 540,000 IU orally had absolute increases of 25OHD levels to greater than 75 nmol/L at day 7, a percentage that remained nearly unchanged to day 28 (20). They also found significant increased $1,25(\text{OH})_2\text{D}$ levels at days 3 and 7 only, and PTH levels were decreased more markedly in the cholecalciferol group than in the placebo group.

No serious adverse effects were observed in their study although isolated levels of 25OHD of greater than 250 nmol/L and ionized calcium of 3 mmol/L were noted. No difference in CRP or procalcitonin was found between the cholecalciferol group and the placebo group. In a study by Leaf et al (21), 67 patients with sepsis were randomized to receive 2 μg of calcitriol ($1,25(\text{OH})_2\text{D}$) or placebo and followed for 48 hours. They found a significant increase in $1,25(\text{OH})_2\text{D}$ at 6 hours, but otherwise no difference in vitamin D metabolites. They found no difference in LL-37 levels or in other markers of inflammation (IL-10, IL-6, tumor necrosis factor- α IL-1b and IL-2) or urinary kidney injury markers over the short study period.

The intramuscular regime used in the current study achieved correction of vitamin D deficiency in the majority of patients by day 7, with levels continuing to rise by day 14. This indicates that intramuscular administration may be a more reliable and effective route of treatment than enteral vitamin D as enteral administration is likely to be less effective for reasons such as gastroparesis and splanchnic ischemia, which are common in critical illness (9).

Strengths and Weaknesses

This study, with the high proportion of patients requiring organ support in the form of mechanical ventilation, vasopressor support, renal replacement therapy, and extracorporeal membrane oxygenation support indicates that complex, critically ill patients were represented.

To date, little is known about the pharmacodynamics of vitamin D replacement in critical illness. Our study examined relationships between vitamin D increments and changes in inflammatory markers. We found that baseline 25OHD levels correlated negatively with IL-6 and positively with LL-37, suggesting an association between higher vitamin D status with an anti-inflammatory/bactericidal cytokine milieu. Also, both CRP and IL-6 levels fell significantly over the study period. We confirmed the observation of Jeng et al (22) that hypovitaminosis D was associated with lower circulating levels of LL-37. We also showed that greater vitamin D increments following supplementation were associated with higher levels of LL-37 in the early phase of critical illness, suggesting potential biological benefits of augmentation of vitamin D levels. This increment dropped away during the recovery phase of illness. This

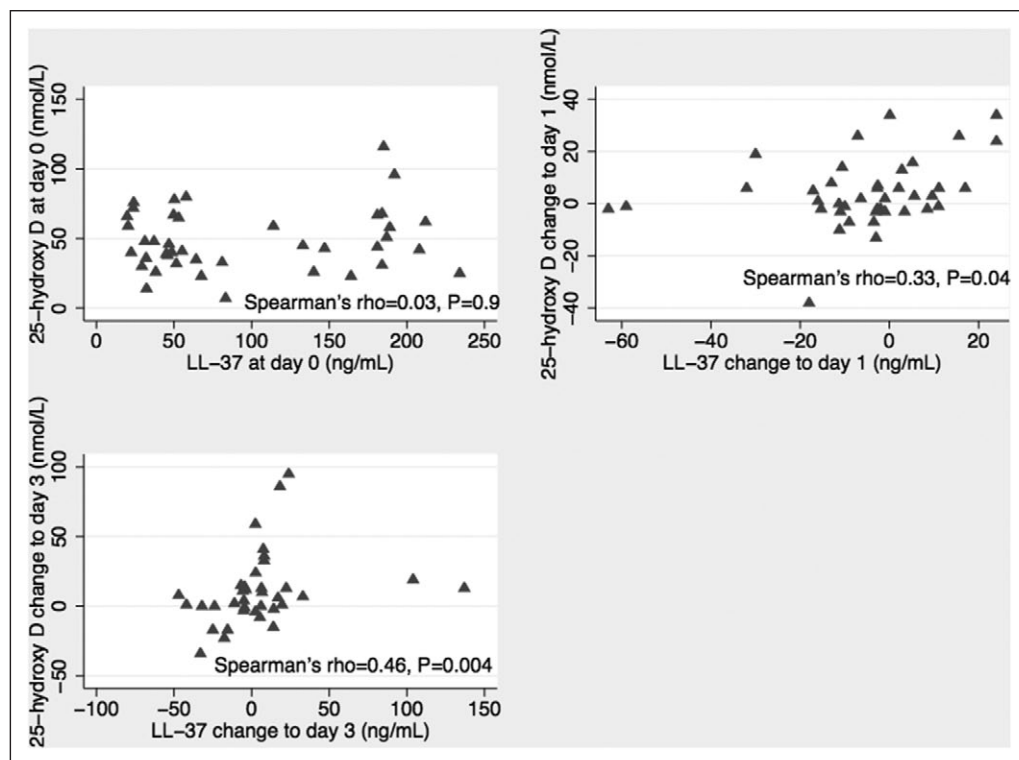


Figure 5. Scatter plots showing the relationship between 25-hydroxy-vitamin D (25OHD) and cathelicidin (LL-37) at day 0 with line of best fit, change in 25OHD and LL-37 from baseline to day 1 and day 3.

observation is supported by a recent report demonstrating a reduction in mortality in severely vitamin D-deficient patients following vitamin D replacement (20).

Our previous study revealed a high prevalence of vitamin D deficiency and secondary hyperparathyroidism in critically ill patients (7). Here, we explored further whether parathyroid status impacts vitamin D repletion. PTH levels showed a trend toward a decrease over the study period with patients achieving vitamin D correction at day 7 having significantly lower PTH levels, independent of renal function.

Importantly, this study did not include a placebo (control) group who did not receive vitamin D; hence, although CRP and

IL-6 levels fell during the study period, this could be a result of clinical improvement over time or the result of other treatments such as antibiotic therapy. However, the primary aim of this study was pharmacokinetic characterization, and we have provided the evidence supporting the efficacy, reliability, and safety of intramuscular vitamin D supplementation.

We did not measure vitamin D-binding protein, free vitamin D, or vitamin D receptor polymorphisms (23) although the implications and clinical relevance of these parameters are not well understood. This study, along with others, has established the foundation to guide future large multi-centre phase III randomized controlled vitamin D inter-

vention trials. The recent VITdAL study has provided important information, including a lower mortality rate in patients with severe vitamin D deficiency, which has been described as hypothesis generating requiring further study (20).

CONCLUSIONS

Vitamin D deficiency is highly prevalent in critically ill patients. Correction of vitamin D deficiency in critical illness is challenging but can be reasonably predictably and safely achieved by a single dose of intramuscular cholecalciferol. Vitamin D repletion is associated with a fall in proinflammatory biomarkers

TABLE 3. Median (Interquartile Range), Minimum, and Maximum Days in ICU and Hospital, by Mortality Status and Randomized Arm

Variable	Low-Dose Vitamin D			High-Dose Vitamin D			<i>p</i> *
	<i>n</i>	Median (IQR)	Min-max	<i>n</i>	Median (IQR)	Min-max	
Patients who survived							
Length of ICU stay	21	14 (7-23)	4-58	20	15 (6.5-28)	2-47	0.93
Length of hospital stay	20	38.5 (22-67.5)	11-90	20	30 (25.5-58)	10-133	0.83
Patients who died							
Days to death for patients dying in ICU	4	14.5 (13-15.5)	12-16	5	24 (10-42)	4-47	0.62
Days to death for patients dying in ICU or after transfer to another ward	5	15 (14-16)	12-52	5	24 (10-42)	4-47	0.92

IQR = interquartile range.

**p* is a comparison of the variable distribution between randomized arms using a Wilcoxon rank-sum test.

TABLE 4. Number (%) of Patients by Outcome Status at 90 Days Post Admission

Status 90 Days Post Admission	Low-Dose Vitamin D	High-Dose Vitamin D	<i>p</i> *
Discharged	19 (76)	17 (68)	0.72
Died	5 (20)	5 (20)	
Inpatient	1 (4)	3 (12)	

**p* value is derived from Fisher exact test.

and greater vitamin D increments were associated with augmented antimicrobial cytokine responses. It is now imperative to investigate the biological and clinical impact of vitamin D repletion in critically ill patients in adequately powered intervention trials.

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